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Relationship between level of antibiotic use and resistance among *Escherichia coli* isolates from integrated multi-site cohorts of humans and swine

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ABSTRACT

The objective of this longitudinal ecological study was to examine the relationship between the prevalence of antibiotic-resistant (AR) commensal *Escherichia coli* isolates from both monthly human wastewater and composite swine fecal samples and the concurrent aggregated monthly antibiotic use recorded within each host species in multi-site vertically integrated swine and human populations. In addition, human vocation (swine worker versus non-swine worker), swine production group, and season were examined as potential confounding variables. Human and swine *E. coli* isolates ($n = 2469$ human and 2310 swine, respectively) were tested for antimicrobial susceptibility using a commercial broth microdilution system. In the human population, among swine workers the relative odds of tetracycline resistance were increased significantly for tetracycline (class) drug use at the third quartile and above of mean monthly dosage (MMD) (OR = 1.8) as compared to the referent category (non-use). The relative odds of ciprofloxacin resistance were significantly increased for ciprofloxacin use in non-swine workers (OR = 5.5) as compared to the referent (non-use). The relative odds of tetracycline resistance were increased significantly for chlortetracycline use in medicated feed for the upper tertile of MMD category (OR = 2.9) as compared to the referent category (no use) across all swine production groups. While high variability among seasonal samples over the 3-year period was observed, no common seasonal trends relating to antibiotic use and prevalence of resistance over the 3-year period were apparent. The overall effects of concurrent human and swine antibiotic use on AR *E. coli* levels were inconsistent and modest in this study.

1. Introduction

Extensive clinical use of antibiotics in both human and veterinary medicine has been blamed for the rise and spread of antibiotic-resistant (AR) bacteria (Levy, 2001). However, non-clinical (e.g., prophylaxis, growth promotion) use of antibiotics in food animals has been suggested as the main driving force behind the extensive propagation and proliferation of AR bacteria, both in animal and human populations (Witte, 1998).

Direct evidence to support the relationship between reduced antibiotic consumption in humans and reduced antibiotic resistance is often lacking. In Finland, investigators reported a considerable drop in the prevalence of erythromycin-resistant *Streptococcus pyogenes* after a

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reduction in macrolide use in humans (Seppala et al., 1997). However, in Australia Stingemore et al. (1989) reported a significant variation in the prevalence of erythromycin resistance in *S. pyogenes* in the absence of any intervention to change macrolide use. Dagan et al. (2008) have shown that reduced antibiotic use during the summer months did not consistently result in a seasonal decrease in AR strains of pneumococci that cause otitis media in children. These investigators showed that among Jewish children <5 years of age in southern Israel, cases of otitis media caused by AR pneumococci were reduced in summer months; perhaps coinciding with a reduction in antibiotic use. However, a similar reduction in otitis media cases associated with AR pneumococci was not seen among Bedouin children. Bedouin children had higher rates of antibiotic use year round, when compared with Jewish children. The authors therefore conclude that the relationship between antibiotic use and the incidence, or proportional morbidity, of cases of clinical disease caused by resistant bacteria is complex.

Similarly, direct evidence to support the relationship between reduced antibiotic use or consumption in animal agriculture and reduced antibiotic resistance is lacking. Investigators have reported associations between levels of antibiotic use (e.g., injectable products, and in medicated feed) and AR bacteria levels in animals (Dunlop et al., 1998a; Funk et al., 2006). However, other studies have suggested that AR bacteria may persist long after the use of antibiotics has been discontinued (Langlois et al., 1988; Bunner et al., 2007), clouding the interpretation of results following cessation of antibiotic use. A precise quantification of the impact on resistance of therapeutic and subtherapeutic (i.e., growth promoters) antibiotic use in livestock production is difficult, in part because of the imprecision of clinical case definitions for livestock diseases, and difficulty in achieving accurate estimates of the actual quantities of injectable and in-feed antibiotics consumed by the animals.

Many of the antibiotic resistance studies that have examined the relationships between AR bacteria and antibiotic use, both at the human and/or animal level, have been cross-sectional in nature (Dunlop et al., 1998b; Mathew et al., 2001; Bunner et al., 2007). Furthermore, other studies have been conducted in open populations with limited or no control over the movement of study subjects in and out of the study area (Stingemore et al., 1989; Tragesser et al., 2006; Jorgensen et al., 2007; Dagan et al., 2008). Knowledge of who is prescribed antibiotics and when and where this occurs is extremely limited in open populations of humans. Instead, it is can be optimal to conduct such studies in relatively stable populations as well as to follow individuals or groups of animals and humans over time to better assess the effects of antibiotic use in relation to AR bacteria.

The objective of this 3-year longitudinal study, conducted in a multi-site and vertically integrated swine and human agri-food system, was to examine the relationship between the prevalence of AR commensal *Escherichia coli* isolated from human wastewater and swine composite fecal samples arising from group-level cohorts of humans and swine and the concurrent antibiotic use by host species (human or swine), while adjusting for the potential confounding effects of human vocation (swine workers versus non-swine workers), swine production group, and season.

2. Materials and methods

2.1. Study design and population

The study system consisted of human and swine populations in multiple units (n = 19 human; n = 12 swine) across the state of Texas. Out of the 19 human units, 12 had co-located swine operations, 6 units had no swine operations, and 1 unit had a swine slaughter plant facility. The human populations were initially classified into 4 groups based on their vocation/housing location as: (1) swine workers, (2) non-swine workers, (3) swine slaughterhouse workers, and (4) non-swine workers/non-system consumers. The first three groups had the potential to consume pork produced within the system, while the fourth group did not. Furthermore, the fourth human group represented the human cohort housed outside of the study system. The movement of new residents into and out of the system was very limited. Only swine workers and non-swine workers data were included in our present analysis since antibiotic use data were not available for either the non-swine workers/non-consumers or the slaughter-plant workers.

Swine populations consisted of 5 farrow-to-finish and 7 grower-to-finisher operations. One farrow-to-finish multiplier unit occasionally purchased (every ~6 months) boars and gilts for breeding purposes. Imported pigs were quarantined (~4 weeks) in a single quarantined unit within the system before they were moved to other farms. Pigs in the study system moved through the system as follows: farrowing barns, hot nursery, cold nursery, grower, finisher barns, lairaged overnight in a holding pen, and last sent to be slaughtered. Pork products were processed at the in-system slaughter plant and fed back to the human populations housed within the agri-food system. Swine populations were categorized, for data analysis purposes, into 7 production groups: (1) farrowing barn sows and piglets, (2) grower and finisher pigs, (3) breeding boars, (4) quarantined boars (purchased boars held at the quarantined unit), (5) breeding/gestation female pigs, (6) nursery piglets (both hot and cold nursery), and (7) pigs in holding pens at the slaughter-plant. Antibiotic use data (i.e., antibiotic records) were not applicable to the pigs in the holding pens; therefore, only the first 6 swine production groups were included in the analysis. The number of samples collected per human vocation (swine worker and non-swine workers) and the 6 swine production groups is shown in Table 1.

2.2. Sampling protocol

Monthly wastewater samples (~50 ml) were collected from all 19 human units over a period of 36 months (February 2004–January 2007). These wastewater (i.e., sewage) samples were collected from manholes known to drain lavatory facilities representative of each of the 4 human groups. Three wastewater samples each from both

Table 1
Number of wastewater and fecal samples collected, and *E. coli* isolated by human vocational cohorts and swine production groups.

No. of *E. coli* isolated and samples collected			
Host-species	Human vocation/ swine production groups	No. of samples collected[a]	No. of *E. coli* isolated[b]
Human	Swine workers	1118	1161
	Non-swine workers	1858	1308
	Total	2976	2469
Swine	Breeding boar	195	157
	Quarantined boar	202	274
	Breeding/gestation females	245	112
	Farrowing sows and piglet	656	528
	Grower and finisher Pig	1371	1043
	Nursery piglets	229	196
	Total	2898	2310

[a] Number of wastewater and fecal samples collected by human vocation and swine production type.
[b] Number of *E. coli* isolated from wastewater and fecal samples by human vocation and swine production type.

swine workers and non-swine workers (included both consumers and non-consumers) were collected monthly at the 12 human units (units with swine operations), in addition to a mixed influent sample (draining from both groups). Three non-swine worker wastewater samples and one mixed influent sample were collected monthly at 6 human units (units with no swine operations). Three wastewater samples from slaughter-plant workers and three from non-swine workers as well as one mixed influent sample were collected at the slaughter-plant unit. Samples were acquired and shipped on ice by a privately licensed company to the USDA-ARS-Southern Plains Agricultural Research Center (USDA-ARS-SPARC, College Station, Texas) for further analysis. For swine, monthly composite fresh fecal floor samples (~50 g) and barn-wash/pre-lagoon influent samples (~50 ml) were collected from pen floors at each of the 12 swine farms and at the slaughter-plant holding pens for a period of 36 months (January 2004–December 2006). Samples were collected and transferred on ice to the USDA-ARS-SPARC laboratory by a swine specialist veterinarian. The composite fecal samples were composed of equal portions of fecal pats collected randomly at each pen. The barn wash/pre-lagoon samples were collected at the multiple points that drained from representative swine pens. In total, across all 12 units approximately 140 composite fecal samples and 35 barn wash/pre-lagoon samples were collected monthly. The number of swine samples collected every month was slightly variable due to the changes in the number of pigs at different production stages within the study system over course of the study. Further details on the study design and study populations as well sampling protocol can be found elsewhere (Alali et al., 2008).

2.3. Antibiotic use data

2.3.1. Human population

Antibiotic records in the human populations were obtained from a centralized computer system that stored all prescriptions dispensed to patients housed within the study system. Antibiotics were prescribed to patients either on an out-patient basis by physicians in a unit clinic or at the in-patient hospitals. At the unit clinic, patients were either required to visit daily to obtain their antibiotics or allowed to carry the medication and use it as prescribed. Every prescription was first entered into a centralized computer system by the attending physician, filled by a central pharmacy within the study system, and then sent to the unit within 24 h. The antibiotic class, duration of use (i.e., treatment), route of administration, daily dose, and records of unused medications returned to the pharmacy by unit clinics and hospitals during the study period, were recorded into the mainframe computer. Antibiotic use records were retrieved for the period between February 2004 and January 2007. The antibiotic use records were aggregated by class (e.g., tetracyclines, fluoroquinolones), unit ($n = 19$) and housing cohort (swine worker or non-swine worker) on a monthly basis ($n = 36$). The records obtained each had the specified antibiotics as the active ingredient. Antibiotic records were unavailable for both the non-swine workers/non-consumers and the slaughter-plant workers.

Prior to delivery to the research team, patient identifiers were stripped from the records for ethical reasons. Only date, unit name and housing type (occupational cohort) were available and utilized to aggregate the consumption data. The mean monthly dosages (MMD) for each of 19 units in the human population were aggregated by month and housing type, and calculated as follows:

$$\mathrm{MMD}_{\mathrm{Human}} = \frac{\text{total amount of antibiotic used by month and vocation cohort (g) in a unit}}{[\text{total number of humans at risk in a category (i.e., swine worker } vs. \text{ non-swine worker) in the unit} \times \text{average body mass of a person at risk (kg)}] \times 1 \text{ month}}$$

The average body weight of a person at risk was obtained from a study conducted by Ogden et al. (2004) from the United States Department of Health and Human Services. The mean mass of men over the age of 20 in the United States, between 1999 and 2002, was 86 kg. The MMD were calculated for the following antibiotic classes: β-lactams (e.g., amoxicillin), fluoroquinolones (e.g., ciprofloxacin), tetracyclines (e.g., tetracycline and doxycycline), and sulfonamides (e.g., sulfadiazine and sulfasalazine).

2.3.2. Swine population

Antibiotics were distributed to the swine populations as both medicated feed (i.e., feed supplemented with antibiotics) and directly administered to individual swine through parenteral routes (i.e., injectable antibiotics). There was no formal antibiotic reporting system for actual use or administration in the individual pigs; therefore, we utilized the monthly listing of feedgrade antibiotic used (via formulations) and monthly antibiotic dispensing records to derive composite values. The antibiotic use records were requested for the period between January 2004 and December 2007. Antibiotic use data were aggregated by antibiotic class, unit, and swine production group. There were no antibiotic use data available on pigs in holding pens at the slaughter-plant. The mean monthly

dosages (MMD) for swine in each production group at each unit were calculated as follows:

$$\mathrm{MMD_{Swine}} = \frac{\text{total amount of antibiotic used by month and production group (g) in a unit}}{[\text{total number of pigs in the unit} \times \text{median body mass of a pig by production type (kg)}] \times 1 \text{ month}}$$

The average body mass of a pig in each swine production group was estimated by a swine specialist veterinarian. The MMD were calculated for the following antibiotic classes: tetracyclines (e.g., chlortetracycline [in-feed] and oxytetracycline (injectable)), cephalosporins (e.g., ceftiofur (injectable)), and florfenicol (injectable).

2.4. Phenotypic analysis of AR E. coli

As human wastewater and fecal samples arrived at the laboratory, aliquots of the samples were frozen at −72 °C with and without 25% glycerol for later analysis. Human wastewater samples with glycerol were thawed, and 1 ml portions were added to 9 ml tryptic soy broth (TSB) broth (Becton, Dickinson and Company, Sparks, MD) and incubated for 18 h at 37 °C for enrichment. The enrichment broth was streaked onto a selective medium of CHROM agar-*E. coli*™ (DRG International, Mountainside, NJ) and incubated further for 18–24 h at 37 °C. Thawed swine fecal samples with glycerol were cultured by directly streaking onto CHROM agar without the aforementioned enrichment step. A single typical *E. coli* colony was randomly selected off the CHROM agar plate, streaked onto a blood agar plate, and then incubated (18 h, 37 °C). After incubation, an *E. coli* isolate was picked from the blood agar and tested for antibiotic susceptibility by the broth microdilution method following the Clinical Laboratory and Standards Institute (CLSI) (formerly NCCLS) standards (NCCLS, 2002) to determine the minimum inhibitory concentration (MIC). Antibiotic susceptibility testing was performed using custom panels designed by the National Antimicrobial Resistance Monitoring System (NARMS) (CDC, 2003) via the Sensititre™ automated system (Trek Diagnostic Systems, Cleveland, OH). The MIC values for the tested *E. coli* isolates were determined to be either resistant or susceptible based on CLSI break points, or else NARMS standards when CLSI guidelines did not provide break-points (i.e., for ceftiofur). MIC results interpreted to be of intermediate susceptibility were reclassified as susceptible. Samples were analyzed on a quarterly basis (i.e., seasonally) over the 3-year study period. All human and swine samples collected during the first 12 months of the study were phenotypically analyzed. These data were later collapsed into 4 seasons (or quarters) based on: (1) winter: February–April, (2) spring: May–July, (3) summer: August–October, and (4) autumn: November–January). Thereafter, only quarterly (i.e., seasonal winter, spring, summer, and autumn) phenotypic analysis was performed on the remaining 24 months of sampling. Quarterly sampling and analysis was conducted because the highest variability was observed between seasons as compared to within season based on the first 12 months of data analysis.

2.5. Statistical analyses

The antibiotic use data, aggregated by month, were further categorized based on the MMD distribution for each antibiotic (Table 2). The antibiotic MMD categories were cross-tabulated with the corresponding antibiotic resistant *E. coli* outcomes (binary) and stratified by each of the following risk factors: (1) host-species (human or swine), (2) human vocation (swine workers versus non-swine workers) or swine production type (e.g., breeding/gestation, breeding boars, farrowing, nursery, grower-finisher, isolation boar, slaughter holding pens), and (3) season-year. The proportion of bacterial isolates resistant to each of the antibiotics was compared across the levels of each antibiotic MMD categories by each risk factor, using either a two-tailed Fisher's exact test or 2-by-*n* likelihood ratio chi-square test, as appropriate, using STATA software version 10.2 (Stata Corp., College Station, TX).

The association between the individual AR *E. coli* phenotypes and the antibiotic class MMD categories, adjusted for host-species, human vocation or swine production group (respectively), and season-year was assessed using a generalized linear model, with binomial error distribution and logit link function and adjusted for dependency of responses within each unit location using a generalized estimated equation (GEE) in STATA version 10.2 software.

Table 2
The distribution and categories of MMD (g/host kg-month) by antibiotic and host-species (human and swine).

Distribution and categories of MMD by antibiotic and host-species[a]									
	MMD distribution					MMD categories			
	Min.	25%	50%	75%	Max.				
Human									
Amoxicillin	0	0.00133	0.00309	0.00624	0.01755	≤0.001	>0.001–≤0.003	0.003–≤0.006	>0.006
Ciprofloxacin	0	0	0	0.00006	0.00220	Non-use		Use	
Tetracycline	0	0.00133	0.00311	0.00523	0.03416	≤0.001	>0.001–≤0.003	0.003–≤0.005	>0.005
Sulfonamide	0	0	0.00142	0.00394	0.03628	≤0.001	>0.001–≤0.004		>0.004
Swine									
Ceftiofur	0	0	0	0	0.06804	Non-use		Use	
Oxytetracycline	0	0	0	0	0.62433	Non-use		Use	
Chlortetracycline	0	0	0	0.04256	0.82826	0	>0–≤0.04		>0.04
Florfenicol	0	0	0	0	0.06189	Non-use		Use	

[a] The antibiotic use (MMD) distribution for human and swine was overall human vocation cohorts and swine production groups, respectively.

The ordinal response (multidrug resistance from 0 to 6+ antibiotics out of a possible maximum of 15) for *E. coli* phenotypes in relation to the antibiotic MMD quantile categories adjusted for host-species, human vocation or swine production group (respectively), and season-year was assessed by using a generalized linear model, with a multinomial distribution and a cumulative logit link function while adjusting for dependency using GEE within each unit location in SAS version 9.1 software (PROC GENMOD; SAS Institute, Inc., Cary, NC). Multidrug resistance outcomes >6 were collapsed with response number 6 (called 6+) because of the rare resistance phenotypes with very sparsely populated cells for some of the higher outcome categories.

3. Results

There were 4779 (2469 human and 2310 swine) commensal *E. coli* isolates collected from the wastewater and fecal matter samples over the 3-year study period and used in this study's analyses. The antibiotic MMD categories are cross-tabulated with antibiotic resistant *E. coli* outcomes (binary) by: (i) human vocation (swine workers versus non-swine workers—see Table 3), and (ii) by swine production type (e.g., breeding/gestation, breeding boars, farrowing, nursery, grower-finisher, quarantine boars—see Table 4).

3.1. Relationship between E. coli resistance to individual antibiotics and antibiotic use in human populations

The relative odds of tetracycline resistance among isolates were significantly increased for tetracycline class use at the third MMD quartile category only (>0.003–≤0.005 g of tetracycline per human kilogram per month (g/human kg-month) isolates (OR = 1.8) as compared to the referent category (MMD '≤0.001' g/human kg-month) in swine workers. However, no significant relationship was observed between tetracycline class use and tetracycline resistance in the non-swine worker group. The relative odds of ciprofloxacin resistance were significantly increased for ciprofloxacin use category (dichotomized as yes or no) (OR = 5.5) as compared to the referent (non-use) category in the non-swine workers. The multivariable statistical model did not converge for the relationship between the ciprofloxacin use and resistance for the swine worker group due to the overall lack of resistant isolates among this group. There was no relationship observed between amoxicillin use or sulfonamide use and amoxicillin/clavulanic acid resistance or sulfisoxazole resistance, respectively, in the human populations. There was no common seasonal trend evident for either antibiotic use or the prevalence of resistance detected over the 3-year period (data not shown).

3.2. Relationship between E. coli resistance to individual antibiotics and antibiotic use in swine populations

The relative odds of tetracycline resistance were significantly increased for chlortetracycline use in medicated feed at the upper MMD tertile category only (>0.04 g/swine kg-month) isolates (OR = 2.9) as compared to the referent category (MMD '=0' g/swine kg-month) isolates across all swine production groups. However, no significant relationship was observed between oxytetracycline

Table 3
Phenotypic resistance of *E. coli* isolates by antibiotic MMD (g/human kg-month) categories use sampled across human vocation cohorts[a].

No. of resistant and total *E. coli* isolates sampled (% of total) by human vocation and antibiotic MMD categories[a]					
Human vocation	Amoxicillin[b]				
	≤0.001	>0.001–≤0.003	0.003–≤0.006	>0.006	*P*-value[c]
Swine worker	7/372 (1.90)	2/243 (0.80)	5/202 (2.50)	4/344(1.20)	0.46
Non-swine worker	2/159 (1.30)	14/412 (3.40)	2/254 (0.80)	10/483 (2.10)	0.1
Human vocation	Ciprofloxacin				
	Non-use	Use	*P*-value		
Swine worker	6 (0.06)	0/129 (0)	0.23		
Non-swine worker	2/671 (0.30)	10/637 (1.60)	0.01		
Human vocation	Tetracycline				
	≤0.001	>0.001–≤0.003	0.002–≤0.005	>0.005	*P*-value
Swine worker	110/429 (25.60)	10/76 (13.20)	68/276 (24.60)	77/380 (20.30)	0.19
Non-swine worker	8/74 (10.80)	30/199 (15.10)	91/452 (20.10)	112/583 (19.20)	0.18
Human vocation	Sulfonamide				
	≤0.001	>0.001–≤0.004	>0.004	*P*-value	
Swine worker	96/871 (11.00)	16/96 (16.70)	18/194 (9.30)	0.04	
Non-swine worker	44/279 (15.80)	71/610 (11.60)	61/419 (14.60)	0.12	

[a] Phenotypic resistance of commensal *E. coli* isolates from human wastewater samples (n = 2469) isolates, with vocation cohorts identified. Frequencies and proportions are contrasted with human vocation cohorts across all unit locations and seasons. MMD = Mean monthly dosage for antibiotics used in this study.

[b] MMD categories consisted of isolates from units with prescribed MMD ≤0.001 (g/human kg-month) for each antibiotic used in the human populations.

[c] *P*-values are based on a likelihood ratio chi-square test of the differences in risk between human MMD categories per antibiotic. These *P*-values are not adjusted for the dependence of responses within unit locations.

Table 4
Phenotypic resistance of *E. coli* isolates by antibiotic MMD (g/swine kg-month) categories use sampled across swine production groups[a].

No. of resistant and total *E. coli* isolates sampled (% of total) by swine production group and antibiotic MMD categories[a]							
Swine production groups	Ceftiofur			Swine production groups	Oxytetracycline		
	Non-use	Use	*P*-value[b]		Non-use	Use	*P*-value
Breeding boar isolates	1/157 (0.60)	0 (0.00)	–	Breeding boar isolates	142/156 (91.00)	1/1 (100.00)	0.67
Quarantined boar isolates	3/274 (1.10)	0 (0.00)	–	Quarantined boar isolates	270/274 (98.50)	0 (0.00)	–
Breeding/gestation female isolates	112/112 (100.00)	0 (0.00)	–	Breeding/gestation females isolates	89/111 (80.20)	1 (0.00)	0.07
Farrowing sows and piglet isolates	7/344 (2.00)	7/184 (3.80)	0.24	Farrowing sows and piglet isolates	275/339 (81.10)	167/189 (88.40)	0.03
Grower and finisher pig isolates	21/1033 (2.03)	0/10 (0.00)	0.52	Grower and finisher pig isolates	761/929 (81.90)	100/114 (87.70)	0.11
Nursery piglet isolates	10/196 (5.10)	0 (0.00)	–	Nursery piglet isolates	169/193 (87.60)	3/3 (100.00)	0.37

Swine production groups	Chlortetracycline				Swine production groups	Florfenicol		
	0	>0–≤0.04	>0.04	*P*-value		Non-use	Use	*P*-value
Breeding boar isolates	100/114 (87.70)	0 (0.00)	43/43 (100.00)	0.002	Breeding boar isolates	1/157 (0.60)	0 (0.00)	–
Quarantined boar isolates	162/164 (98.80)	0 (0.00)	108/110 (98.20)	0.69	Quarantined boar isolates	35/274 (12.80)	0 (0.00)	–
Breeding/gestation females isolates	87/109 (79.80)	0 (0.00)	2/3 (66.70)	0.60	Breeding/gestation females isolates	3/112 (2.70)	0 (0.00)	–
Farrowing sows and piglet isolates	357/440 (81.10)	0 (0.00)	85/88 (96.60)	<0.001	Farrowing sows and piglet isolates	13/528 (2.50)	0 (0.00)	–
Grower and finisher pig isolates	387/475 (81.50)	271/339 (79.90)	203/229 (88.70)	0.01	Grower and finisher pig isolates	27/1043 (2.60)	0 (0.00)	–
Nursery piglet isolates	25/31 (80.70)	38/42 (90.50)	109/123 (88.60)	0.44	Nursery piglet isolates	7/178 (3.90)	1/18 (5.60)	0.75

[a] Phenotypic resistance of commensal *E. coli* isolates among swine fecal samples ($n = 2351$ isolates, with production groups identified). Frequencies and proportions are contrasted by swine production groups across all unit locations and seasons.

[b] *P*-values are based on a likelihood ratio chi-square test of the differences in risk between swine MMD (g/swine kg-month) categories per antibiotic. These *P*-values are not adjusted for the dependence of responses within unit locations. *P*-value = '–' due to lack of resistant isolates in at least one of the MMD categories.

(injectable) use and tetracycline resistance in the swine isolates. Furthermore, no relationship was observed between injectable ceftiofur or florfenicol use and ceftiofur or chloramphenicol resistance, respectively, in the swine populations. We did not observe a common seasonal trend between antibiotic use and the prevalence of resistance over the 3-year period (data not shown).

3.3. Relationship between E. coli resistance to multiple antibiotics and antibiotic use in human populations

The multinomial ORs for the total multidrug resistance phenotype in relation to each of the antibiotic class measures, adjusted for the dependence of human isolate response within each unit location, did not differ significantly ($P > 0.05$).

3.4. Relationship between E. coli resistance to multiple antibiotics and antibiotic use in swine populations

The multinomial ORs for the total multidrug resistance phenotype in relation to the antibiotic use, adjusted for the dependence of swine isolate response within each unit location, were significantly ($P < 0.05$) increased among chlortetracycline (in-feed antibiotic) use only for the third tertile MMD category (>0.004 g/swine kg-month) isolates (OR = 1.51 [95% CI, 2.0–13.7]) compared to those of the referent (MMD '=0' g/swine kg-month) across all levels of multidrug resistance.

4. Discussion

To the best of our knowledge, the present study is the first to longitudinally assess the varying prevalence of AR bacteria in integrated human and swine populations in relation to levels of human and swine antibiotic use.

The relative odds of tetracycline resistance were significantly increased for tetracycline class use at the third (out of four) MMD quantile category (>0.003–≤0.005 g/human kg-month) isolates (OR = 1.8) as compared to the referent category in swine workers, but no significant differences were detected in the non-swine worker MMD categories. However, the proportion of non-workers who were prescribed antibiotics belonging to the tetracycline class was higher than the proportion of swine workers. A possible explanation for the higher odds of resistance in the third MMD category is that swine workers are in contact with swine medicated feed which commonly contains large amounts (i.e., high levels) of subtherapeutic chlortetracycline. Nijsten et al. (1994) have shown in The Netherlands that pig farmers harbored significantly more resistant *E. coli* isolates than did suburban and urban

residents. The authors suggested that these findings might be a result of direct exposure of farmers to antibiotics, for example, when in contact with medicated pig feed. Farmers also might involuntarily ingest antibiotics via handling or through inhaling dust that contains swine medicated feed (Nijsten et al., 1994).

The relative odds of ciprofloxacin resistance among isolates were significantly increased for ciprofloxacin use (OR = 5.5) as compared to the referent category (non-use) isolates in the non-swine workers. Ciprofloxacin belongs to the fluoroquinolone class of drugs. The findings in our study are in agreement with a study conducted in Spain by Garau et al. (1999). Those authors showed that prior exposure to quinolones was significantly associated (OR = 14) with quinolone-resistant *E. coli* isolates. However, because of the extremely rare nature of quinolone resistance in our study, the multivariable (adjusted) models were unstable. There was no relationship observed between amoxicillin use and amoxicillin/clavulanic resistance or sulfonamide use and sulfisoxazole resistance in the human populations. Oteo et al. (2008) reported an increase in amoxicillin/clavulanic acid resistant *E. coli* levels which coincided with growing levels of amoxicillin/clavulanic acid consumption at the community level.

The relative odds of tetracycline resistance were significantly increased for chlortetracycline use in medicated feed at the upper MMD tertile category (>0.04 g/swine kg-month) as compared to the referent (no use) across swine production groups. This is in agreement with other studies in which authors reported a relationship between tetracycline resistant *E. coli* isolates from swine and levels of tetracycline use (Dunlop et al., 1998b; Mathew et al., 2001; Dewulf et al., 2007). In contrast, Akwar et al. (2008) reported no statistically significant association between tetracycline use (in-feed and injectable) and resistant *E. coli* in 47 swine farms in Canada.

There was no significant relationship observed between oxytetracycline (injectable) use, ceftiofur use, or florfenicol use, and tetracycline resistance, ceftiofur resistance, or chloramphenicol resistance, respectively, in the swine population. The use of these 3 antibiotics was rare in the swine farms we studied. These treatments also were applied to individual pigs, whereas in-feed medications blanketed all pigs and the net effect may have been greater. The nature of our samples (composite) would likely dilute the effects expected to be observed at the individual level, but are useful for ecological interpretations of populations of bacteria colonizing populations of animals and humans. Oxytetracycline was used mainly in units containing farrowing sows and piglets and breeding/gestation females. Oxytetracycline was used primarily to treat bacterial enteritis and pneumonia in pigs. O'Connor et al. (2002) did not detect significant additional effects of the oxytetracycline injectable use on the prevalence of tetracycline resistance in beef cattle. This finding may have been due to the short-term effect of the injectable drug on tetracycline resistance, as discussed by those authors. Ceftiofur was mainly used in swine units containing farrowing sows and piglets, breeding/gestation female pigs, and to a lesser extent in grower and finisher pigs and nursery piglets. Ceftiofur was mainly used to treat piglet diarrheal diseases caused by *E. coli*. The prevalence of ceftiofur resistant *E. coli* isolates from the swine population was low (2.1%). In contrast to our study findings, Jorgensen et al. (2007) reported a statistically significant association between the use of ceftiofur and reduced susceptibility to cefotaxime (third generation cephalosporin drug) in Danish swine farms. However, those authors used individual animal fecal samples and not composites. In addition, associations between ceftiofur use and isolation of *E. coli* with reduced susceptibility to ceftriaxone have been reported in dairy cattle farms (Tragesser et al., 2006). Moreover, in a clinical trial Lowrance et al. (2007) reported higher proportions of ceftiofur-resistant *E. coli* isolated from feedlot cattle treated with ceftiofur compared to controls in a field trial. Florfenicol was mainly used in nursery piglets to treat central nervous diseases such as *Streptococcus suis* type 2 meningitis (personal communication with swine specialist veterinarian). The prevalence of chloramphenicol resistant *E. coli* isolates from the swine population was 3.8%. To the best of our knowledge, there are no other published reports on chloramphenicol resistant *E. coli* bacteria levels in relation to florfenicol use in swine.

We did not observe a common seasonal trend relating antibiotic use and the prevalence of resistance among swine and human samples over the 3-year period. We attempted time-series analysis to determine the seasonal trend in our longitudinal data. The time-series models did not fit the data well and that is likely because our data are binary in nature which makes it difficult to analyze using time-series models (time-series models best explain the trends associated with time for continuous data).

In conclusion, the present study is the first to longitudinally assess the prevalence of AR bacteria in aggregated human and swine populations in an integrated agri-food system in relation to the aggregated indices of human and swine antibiotic use. Therapeutic use of antibiotics in the human populations was not associated with antibiotic resistant *E. coli* isolates except for tetracycline class use (in one MMD category) and fluoroquinolone class (i.e., ciprofloxacin) use category (dichotomized). In swine, therapeutic use of antibiotics was not associated with differences in prevalence proportions of antibiotic resistant *E. coli* isolates. However, chlortetracycline in feed was associated with an increased prevalence of tetracycline resistant *E. coli* swine isolates. Antibiotic use alone does not generally explain the spread and persistence of AR bacteria in aggregated animal populations in which the antibiotic use is discontinued or else is very sporadic or limited. Other risk factors such as antibiotic residues in the environment (e.g., soil, water, wastewater, and lagoons) and AR gene dissemination in different human and animal populations also have an impact on the AR levels on farms and consequently on the food chain. Aggregated and mass-population exposure to antibiotics appears to have a greater impact on detectable differences in resistance measured at the population level, as witnessed in our study system swine populations. Individual-level treatments, where the effect is diluted out among the many non-treated individuals in the same population, may not yield the same contemporaneous measurable effect at the ecological aggregated level.

Therefore, based on the results of this study, it seems that mass antibiotic medication (i.e., feed supplemented with antibiotics) in animal populations has a greater impact on the selection pressure toward presence of AR bacteria in animal cohorts compared to individual-level treatments.

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